U.S. DEPARTMENT OF COMMERCE PATENT & TRADEMARK OFFICE

B/O Form PTO-1390 **Transmittal Letter to the United States Designated/Elected Office (DO/EO/US) Concerning a Filing Under *35 USC 371***		*Attorney's Docket Number* SCHR3002/REF
		U.S. Application Number (if known) 09/926002
International Application Number PCT/EP00/01038	*International Filing Date* 9 February 2000	*Priority Date Claimed* 12 February 1999
Title of Invention VACCINE FORMULATION		
Applicant(s) for DO/EO/US SCHRÖDER et al.		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items under *35 USC 371*:

1. ☒ This is a **FIRST** submission of items concerning a filing under *35 USC 371*.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under *35 USC 371*.

3. ☒ This express request to begin national examination procedures *(35 USC 371(f))* at any time rather than delay examination until the expiration of the applicable time limit set in *35 USC 371(b)* and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed *35 USC 371(c)(2)*.
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English *(35 USC 371(c)(2))*.

7. ☒ Amendments to the claims of the International Application under PCT Article 19 *(35 USC 371(c)(3))*
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 *(35 USC 371(c)(3))*.

9. ☐ An oath or declaration of the inventor(s) *(35 USC 371(c)(4))*. (☐ Executed ☐ Unexecuted)

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 *(35 USC 371(c)(5))*.

Items 11 to 16 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under *37 CFR 1.97* and *1.98*.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with *37 CFR 3.28* and *3.31* is included.

13. ☒ A **FIRST** preliminary amendment.
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: Applicant asserts claim for small entity.

Application Number (if Known)	International Application Number	Attorney's Docket Number
09/926002	PCT/EP00/01038	SCHR3002/REF

	Calculations	PTO USE ONLY
17. The following fees are submitted: **Basic National Fee** *(37 CFR 1.492(a)(1)-(5))*: ☒ Search report has been prepared by the EPO or JPO . . . . . $860.00 ☐ International Preliminary Examination Fee paid to USPTO *(37 CFR 1.482)* . . . . . $690.00 ☐ No International Preliminary Examination Fee paid to USPTO *(37 CFR 1.482)* but International Search Fee paid to USPTO *(37 CFR 1.445(a)(2))* . . . . . $710.00 ☐ Neither International Preliminary Examination Fee *(37 CFR 1.482)* nor International Search Fee *(37 CFR 1.445(a)(2))* paid to USPTO . . . . . $1000.00 ☐ International Preliminary Examination Fee paid to USPTO *(37 CFR 1.482)* and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	$860.00	
ENTER APPROPRIATE BASIC FEE AMOUNT	**$ 860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date *(37 CFR 1.492(e))*.		

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	10 -20 =	0	× $18.00	$ 0.00	
Independent Claims	1 -3 =	0	× $80.00	$ 0.00	
Multiple Dependent Claims (if applicable)			+ $270.00		
TOTAL OF ABOVE CALCULATIONS				**$ 860.00**	
Reduction by ½ for filing by small entity, if applicable. Small Entity Status is asserted pursuant to 37 CFR 1.27 for this application.				$ 430.00	
SUBTOTAL				**$ 430.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date *(37 CFR 1.492(f))*.					
TOTAL NATIONAL FEE					
Fee for recording the enclosed assignment *(37 CFR 1.21(h))*. The assignment must be accompanied by an appropriate cover sheet *(37 CFR 3.28, 3.31)*. **$40.00** per property.					
TOTAL FEES ENCLOSED				**$ 430.00**	
Amount to be:				Refunded:	
				Charged:	

a. ☒ A check in the amount of **$430.00** to cover the fees is enclosed.

b. ☐ Please charge my **Deposit Account Number 02-0200** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to **Deposit Account Number 02-0200**. A duplicate copy of this sheet is enclosed.

Note: Where an appropriate time limit under *37 CFR 1.494* or *1.495* has not been met, a petition to revive *(37 CFR 1.137(a) or (b))* must be filed and granted to restore the application to pending status.

BACON & THOMAS, PLLC
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DATE: August 13, 2001

Respectfully submitted,

Richard E Fichter

Richard E. Fichter
Attorney for Applicant
Registration Number: 26,382

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SCHRÖDER et al.

U.S. National Phase of PCT/EP00/01038

Entry papers filed herewith August 13, 2001

For: VACCINE FORMULATION

Attention: PCT OFFICE

PRELIMINARY AMENDMENT
AND INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The present application is the U.S. national phase of international application number PCT/EP00/01038. The following amendments pertain to the claims as amended.

Please note that the amended pages attached to the International Preliminary Examination Report (Annexes) and submitted herewith, have replaced the originally filed pages 11-12 of the application. The claims to be examined and amended by this preliminary amendment are found on the amended pages after pages 11-12.

Please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please add the attached ABSTRACT OF THE DISCLOSURE to the application.

IN THE CLAIMS:

Please replace claims 3-6 and 8-10 with the following amended claims.

3(Amended). Vaccine formulation according to claim 1, wherein the adjuvant has a monoglyceride preparation content of at least 90%, preferably at least 95%, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more unsaturated bonds, and the immunologically active carriers (IAC) are derived from polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

4(Amended). Vaccine formulation according to claim 1, which further comprises pharmaceutical excipients selected from the group consisting of biocompatible oils, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

5(Amended). Vaccine formulation according to claim 3, wherein the adjuvant is a mixture of mono-olein and oleic acid, and possibly soybean oil, and the immunizing component is lipoarabinomannan-tetanus toxoid (LAM-TT).

6(Amended). Vaccine formulation according to claim 1, wherein the formulation is formulated into a preparation for mucosal administration.

8(Amended). Aerosol or spray package comprising a tuberculosis vaccine composition according to claim 1.

9(Amended). Nose-drop package comprising a tuberculosis vaccine composition according to claim 1.

10(Amended). A method of vaccinating a mammal against a mycobacterium having antigenically active carbohydrate moieties (ACM) derived from *Mycobacterium*

tuberculosis, which comprises mucosal administration to the mammal of an protection-inducing amount of a tuberculosis vaccine composition according to claim 1.

REMARKS

Applicants have amended the claims in order to reduce the initial filing fee by deleting the multiple dependent claims from the application. Applicants retain the right to reintroduce any subject matter canceled by the present Amendment at any time during the prosecution of this application or any further application claiming benefit of this application.

Applicants have amended the application to substitute the originally filed pages 11-12 with the amended pages attached to the International Preliminary Examiner Report (Annexes) and included in the application as filed herewith. Also, an Abstract of the Disclosure has been added to the application.

Applicants are submitting herewith a copy of the Search Report which issued on International Application No. PCT/EP00/01038, of which the present application is the U.S. national phase. All of the publications cited in the International Search Report are listed on the attached Form PTO-1449. It is Applicants' understanding that, under the procedures of the PCT, copies of the cited publications will have been supplied to the U.S. Patent Office by the International Bureau. However, the Examiner is invited to contact the undersigned attorney if additional copies are necessary or would facilitate examination of the present application.

Otherwise, the Examiner is respectfully requested to return an initialed and dated copy of the attached Form PTO-1449 to confirm that all publications listed thereon have been considered and made officially of record in the file of this application.

Applicants understand that, under the procedures of the PCT, a copy of the priority document (SE 9900496-2, filed 12 February 1999) will have been supplied to the U.S. Patent Office pursuant to Rule 17 of the PCT Regulations. It is therefore respectfully requested that the first Official Action in the present application contain an indication that the appropriate priority document is in the file of this application.

In view of the above amendments, an early action on the application is now in order and is most respectfully requested.

Respectfully submitted,
BACON & THOMAS, PLLC

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PA01.wpd

DATE: August 13, 2001

Marked-Up Version Showing Changes Made

IN THE CLAIMS:

Please replace claims 3-6 and 8-10 with the following amended claims.

3(Amended). Vaccine formulation according to claim 1 [or 2], wherein the adjuvant has a monoglyceride preparation content of at least 90%, preferably at least 95%, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more unsaturated bonds, and the immunologically active carriers (IAC) are derived from polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

4(Amended). Vaccine formulation according to [any one of claims 1-3] claim 1, which further comprises pharmaceutical excipients selected from the group consisting of biocompatible oils, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

5(Amended). Vaccine formulation according to claim 3 [or 4], wherein the adjuvant is a mixture of mono-olein and oleic acid, and possibly soybean oil, and the immunizing component is lipoarabinomannan-tetanus toxoid (LAM-TT).

6(Amended). Vaccine formulation according to [any one of claims 1-5] claim 1, wherein the formulation is formulated into a preparation for mucosal administration.

8(Amended). Aerosol or spray package comprising a tuberculosis vaccine composition according to [any one of claims 1-7] claim 1.

9(Amended). Nose-drop package comprising a tuberculosis vaccine composition according to [any one of the claims 1-6] claim 1.

10(Amended). A method of vaccinating a mammal against a mycobacterium having antigenically active carbohydrate moieties (ACM) derived from *Mycobacterium tuberculosis*, which comprises mucosal administration to the mammal of an protection-inducing amount of a tuberculosis vaccine composition according to [any one of claims 1-7] claim 1.

VACCINE FORMULATION

The present invention relates to a novel vaccine formulation against a microorganism e.g. *Mycobacterium tuberculosis*. The preferred route of administration is via the mucosal membranes.

BACKGROUND

The earliest described immunization attempts were carried out in China over 900 years ago, where intranasal inoculation of dried and ground smallpox pustules was performed. In the classical immunology and in combination with vaccination against different types of infectious agents e.g. bacteria, virus or parasites the prevailing dogma has been to administer the vaccine subcutaneously or intramuscularly. However, research has during the last years shown that the body has a very effective immunological system that resides in the mucosa. It has also been shown that you can administer vaccines nasally, orally, rectally and vaginally. In the same way as for the classical immunization it has been shown that by mucosal vaccination there is also a need for enhancement of the immunological response by the addition of adjuvants.

The intranasal route has attracted increased attention because of the greater efficacy in inducing mucosal immune responses than the more conventional regimes of parenteral immunization. Furthermore, the realization that approximately 80% of the immune system reside in the mucosa combined with the fact that an equal percentage of the known pathogens enter our bodies via the mucosal membranes has pushed the interest towards the application of mucosal immunization.

It has also been shown that parenteral vaccines do not induce immune response at mucosal sites. Thus, it is also clear that appropriate stimulation of a mucosal site such as the nose or the gut, can generate immune response at other mucosal sites. As an example, it is possible to apply a vaccine in the nose and obtain an immune response in the vagina. Furthermore, the mucosal immune response is very rapid with onset only hours after being subjected to stimulation by a pathogen, as compared to parenteral immunity having a response time of several days.

Tuberculosis (TB) is one of the major causes of morbidity in the world with an estimated death toll of approximately 3 millions per year. It is estimated that 1/3 of the world's population is infected with TB. To a large extent TB is essentially an

uncontrolled problem despite the use of the Bacille Calmette-Guérin (BCG) vaccine for more than 75 years.

The BCG vaccine consists of a weakened strain of a tuberculosis bacteria taken from a cow in 1908. The original bacteria used today were cultured for 13 years for the purpose of weaken their pathogenic characteristics in order to be used as live bacteria for parenteral vaccination of humans. Basically the same strain is used today as the only vaccine available against TB. Several pharmaceutical companies around the world produce the BCG vaccine. The BCG formulation used today consists of freeze-dried attenuated viable BCG vaccine in one container and another container with physiologically acceptable suspension media. Before administration, the freeze-dried BCG is suspended and subsequently administered by injection to the patient. This procedure which has to be carried out immediately in connection with the vaccination, requires skilled personnel and decent facilities in order to avoid contamination. Unfortunately these criteria are hard to keep up with in the developing countries. Thus, it is estimated that failure to keep to this standard costs about USD 500 millions each year world-wide. Consequently, huge savings could be made both in money and product safety, if a system was available where no mixing of vaccines was needed and where injections could be eliminated, thus eliminating the need for highly skilled personnel and sterile conditions.

In clinical trails around the world, the protective efficacy of the BCG vaccine has been shown to vary between -50% to +80%. This means that certain clinical studies have shown that in fact you enhance instead of diminish your risk of getting the disease after vaccination.

The BCG vaccine works well for children but has more or less no effect on adults. Consequently there are great efforts made in order to achieve a vaccine against TB for the grown-up population. Up to date however, there are no reports in the literature of a TB vaccine that is better than BCG.

Tuberculosis is spread by close person-to-person contact through infectious aerosols. On rare occasions the disease can be acquired by ingestion or skin trauma. This means that the first organ to get into contact with the bacteria during a normal infection is the mucosal surfaces in the lungs.

Adjuvants are a heterogeneous group of substances that enhance the immunological response against an antigen that is administered simultaneously.

Almost all adjuvants used today for enhancement of the immune response against antigens are particles or are forming particles together with the antigen. In the book "Vaccine Design - the subunit and adjuvant approach" (Ed: Powell & Newman, Plenum Press, 1995) almost all known adjuvants are described both regarding their immunological activity and regarding their chemical characteristics. As described in the book more than 80% of the adjuvants tested today are particles or polymers that together with the antigens (in most cases proteins) are forming particles. The type of adjuvants that are not forming particles are a group of substances that are acting as immunological signal substances and that under normal conditions consist of the substances that are formed by the immune system as a consequence of the immunological activation after administration of particulate adjuvant systems.

Using particulate systems as adjuvants, the antigens are associated or mixed with or to a matrix, which has the characteristics of being slowly biodegradable. Of great importance using such matrix systems are that the matrices do not form toxic metabolites. Choosing from this point of view, the main kinds of matrices that can be used are mainly substances originating from a body. With this background there are only a few systems available that fulfill these demands: lactic acid polymers, poly-amino acids (proteins), carbohydrates, lipids and biocompatible polymers with low toxicity. Combinations of these groups of substances originating from a body or combinations of substances originating from a body and biocompatible polymers can also be used. Lipids are the preferred substances since they display structures that make them biodegradable as well as the fact that they are the most important part in all biological membranes.

Lipids are characterized as polar or non-polar. The lipids that are of most importance in the present invention are the polar lipids since they have the capacity to interact and form particulate systems in water. Another way of defining these lipids are as amphiphilic due to their chemical structure with one hydrophobic and one hydrophilic part in the molecule thereby being useable as surface active substances. Examples of main groups of polar lipids are mono-glycerides, fatty acids, phospholipids and glycosphingolipids. These main groups can be further characterized depending on the length of the acyl chain and the degree of saturation of the acyl chain. Since the number of carbon atoms in the acyl chain can be in the range of 6 to 24, and the number of unsaturated bonds can be varied, there is an almost infinite number of combinations regarding the chemical composition of the lipid.

Particulate lipid systems can be further divided into the different groups as discussed in the scientific literature such as liposomes, emulsions, cubosomes, cochleates, micelles and the like.

In a number of systems the lipids may spontaneously form, or can be forced to form, stabile systems. However, under certain circumstances other surface-active substances have to be introduced in order to achieve stability. Such surface-active systems can be of non-lipid character but possess the characteristics of the polar lipids having hydrophobic and hydrophilic parts in their molecular structure.

Another factor that has been shown to be of importance is that lipids exhibit different physical chemical phases, these phases have in different test systems been shown to enhance uptake of biological substances after administration to mucous membranes. Examples of such physical chemical phases described are L2, lamellar, hexagonal, cubic and L3.

In the same way as within the classical immunology where vaccines (antigens) are administered parenterally, there is within mucosal immunization a great interest in directing the immunological response towards development of humoral and/or cellular response. If you obtain a humoral response it would be important to direct the response in a way that a certain class of antibodies would be obtained. In order to obtain such a goal, specific immune stimulating agents can be added to the formulation of antigens and adjuvants.

A formulation which fulfils these goals is described in PCT/SE97/01003, the contents of which is incorporated herein by reference. The disclosed formulation comprises monoglycerides and fatty acids. The monoglycerides comprise one or more substances selected from monoglycerides wherein the acyl group contains from 6 to 24 carbon atoms, preferably 8 to 20 carbon atoms, even more preferably 14 - 20 carbon atoms and where the acyl chain may contain unsaturated bonds.

The acyl chain of the fatty acid may be varied between 4 and 22, preferably 8 to 18 and where the acyl chain may contain one or more unsaturated bonds. A combination of the monoglyceride mono-olein and oleic acid has shown to be an L3 phase, which can be described as sponge-like structure, in contrast to liposomes that form onion-like lamellar structures.

Said combination of monoglycerides and fatty acids my be further formulated by the addition of a biocompatible and biodegradable oil thus forming an oil in water

(o/w) or w/o/w emulsion. Such emulsions have been shown in the literature to be very effective in enhancing the cellular response against an antigen after administration to an animal (Singh, M., et al 1997, *Vaccine* 15, 1773-78). It is generally accepted that in order to have an acceptable vaccine against TB there is a need for a cellular immune response.

Thus, there is a need for a simple way of administering a vaccine combined with an antigen that is easily documented and formulated. One way of producing such a system would be to use antigenic surface components from bacteria which would have the capacity to provoke an immune response in a body, preferably producing a protective immunity against the pathogen which was the origin of the antigen. A number of such systems are available today, however the majority of these are based on membrane components which are proteins.

Most virulent bacteria have carbohydrates on their surface, such as lipopolysaccharides and capsular polysaccharides. Antibodies directed against capsular polysaccharides provide, among other things, enhanced phagocytosis and killing of bacterial cells. Usually there are a number of serotypes of a given bacterial species, for example there are more than 80 known serotypes of Streptococcus pneumoniae related to their carbohydrate capsular structures.

Bacterial polysaccharides are classical examples of antigens that are not T helper cell-dependent, and hence, if they are immunogenic at all, they mainly induce IgM class of antibodies. This is so, because only B cells respond to them, and B cells cannot mediate the memory function as opposed to the T cells, which also mediate immunological booster effects.

In immunologically immature small children, elderly and immunosuppressed persons polysaccharides are known to be poor immunogens or not at all immunogenic.

Therefore, polysaccharide antigens which are chemically conjugated to carriers comprising T cell epitopes are effective as vaccines also for the above mentioned immunologically immature children and immunosuppressed adults.

The vaccine-producing industry has long been searching for a general method of producing conjugate-type vaccines. A general and simple method to produce such vaccines would not only be more practical but would also make process and quality control easier.

A method which provides a general method of producing immunogenic products comprising antigenically active carbohydrate moieties and immunologically active carriers, producing useful immunizing components in conjugate-type vaccines is described in WO 97/ 35613, the contents of which is incorporated herein by reference.

In said International application is described a method of producing an immunogenic product consisting of antigenically active carbohydrate moieties which are each covalently coupled via identical specified divalent bridge groups to immunologically active carriers containing amino groups.

In a preferred embodiment of said invention, the antigenically active carbohydrate moieties of the immunogenic products derive from bacterial O-polysaccharides and/or capsular polysaccharides. Specific examples of such saccharides are those which derive from Salmonella serotypes BO and/or DO or from different serotypes of Streptococcus pneumoniae capsular polysaccharides or from Haemophilus influenzae capsular polysaccharides.

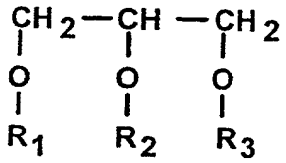
Another carbohydrate moiety, which is of great importance, is the surface carbohydrate from *M. tuberculosis*. This carbohydrate consists of Lipoarabinomannan (LAM) and is the antigenically dominating surface antigen of mycobacteria, and accounts for up to 15 mg/g of the bacterial weight. LAM has profound biologic effects; hence LAM has been reported to interfere with gamma-interferon mediated activation of macrophages, scavenge toxic oxygen free radicals, inhibit protein kinase activity, and induces the expression of macrophage-early genes. LAM has been tested extensively as a possible antigen in vaccine formulations for a long time, however only by parenteral administration, resulting in poor protection in animal experiments.

The immunologically active carriers of the immunogenic product of the conjugate of said invention is preferably derived from polypeptides. In a preferred embodiment said polypeptide is tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

Description of the invention

The present invention is directed to a vaccine formulation against a microorganism comprising, as adjuvant, one or more substances selected from

a) monoglyceride preparations having at least 80 % monoglyceride content and having the general formula



wherein R_1 and R_2 is H and R_3 is one acyl group containing from 6 to 24 carbon atoms, and where the acyl chains may contain one or more unsaturated bonds and

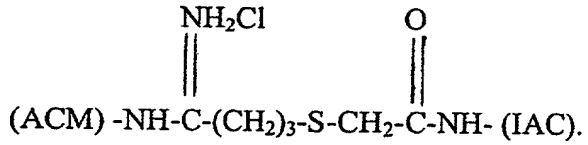
b) fatty acids of the general formula

$$CH_3 - (CH_2)_n - COOH$$

where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and

as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from said microorganism which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC).

In an embodiment of the invention the immunologically active carriers (IAC) contain amino groups and said divalent bridge group has the following structural formula



The adjuvant of the vaccine formulation of the invention preferably has a monoglyceride preparation content of at least 90 %, preferably at least 95 %, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more unsaturated bonds, and the immunologically active carriers (IAC) are derived from polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

The vaccine formulation according to the invention may further comprise pharmaceutical excipients selected from the group consisting of biocompatible oils, such as such as rape seed oil, sunflower oil, peanut oil, cotton seed oil, jojoba oil,

squalan or squalene, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

A most preferred embodiment of the invention is a vaccine formulation which comprises, as adjuvant, a mixture of mono-olein and oleic acid, and possibly soybean oil, and, as immunizing component, lipoarabinomannan-tetanus toxoid (LAM-TT).

Examples of other antigenically active carbohydrate moieties of the immunogenic products of the immunizing component of the invention derive from bacterial O-polysaccharides and/or capsular polysaccharides. Specific examples of such saccharides are those which derive from Salmonella serotypes BO and/or DO or from different serotypes of Streptococcus pneumoniae capsular polysaccharides or from Haemophilus influenzae capsular polysaccharides.

In another preferred embodiment of the vaccine formulation according to the invention, the formulation is formulated into a preparation for mucosal administration, such as nasal, pulmonary, oral, rectal or vaginal administration.

Another aspect of the invention is directed to an aerosol or spray package comprising a TB vaccine composition according to the invention.

Yet another aspect of the invention is directed to a nose-drop package comprising a TB vaccine composition according to the invention.

A further aspect of the invention is directed to a method of vaccinating a mammal against a microorganism having antigenically active carbohydrate moieties (ACM), which comprises mucosal administration to the mammal of an protection-inducing amount of a TB vaccine composition according to the invention.

As described above the present commercially available vaccine against TB comprises an attenuated strain of the bacteria. Such systems may under certain circumstances, when administered as a vaccine, result in an infection by the attenuated bacteria. Furthermore, antigen systems based on whole bacteria are difficult to standardize according to pharmaceutical regulations. Thus, the preferred system as described in the present invention is a purified antigen from the pathogen, which, in combination with adequate adjuvants results in protective immunity. However, certain antigens, such as carbohydrates, may not generate protective immunity if not associated to a carrier in the form of a conjugate. Furthermore, conjugate vaccines are more stable

and consequently more attractive as antigens/vaccines, especially in the developing world.

The present invention describes a formulation that may be prefabricated, and therefore no need for skilled personnel is needed upon nasal administration, thereby eliminating injection systems such as needles and syringes which in developing world often are contaminated and thus is spreading diseases between patients. Furthermore, a device for multidose aerosol delivery of a nasal vaccine can easily be constructed in way that no person-to-person infection can occur.

The invention will now be illustrated by way of an example, which, however, is not to be interpreted as limitation to the scope of protection according to the appended claims.

Short description of the drawing

Fig. 1 shows the results of the testing disclosed in Example 1.

EXAMPLE 1

Protection of C57BL mice from intranasal sub-lethal challenge with *M. tuberculosis* (MT) by immunization with live BCG and heat-killed BCG or lipoarabinomannan-tetanus toxoid (LAM-TT) conjugate in two different L3 lipid adjuvant formulations.

The emulsion was produced by mixing the LAM-TT conjugate with 100 µl of soybean oil and 100 µl of a mixture of mono-olein and oleic acid (1:1). The amount of LAM-TT conjugate was adjusted so that a dose of 10 µg was given to the mice in 100 µl (parenteral) or in 10 µl (nasal). This mixture was sonicated briefly for a few seconds whereupon 1.0 ml of 0.1 M TRIS buffer and 20 µl of 4 M NaOH were added. Sonication was performed for 2 minutes whereupon the emulsion was used for immunization.

An L3 suspension was produced from a 1:1 molar mixture of mono-olein and oleic acid (1.43 g mono-olein and 1.12 g oleic acid) which was added to 40 ml of 0.1 M Tris buffer. Before sonication for 2 minutes 640 µl of 4 M NaOH was added. Before immunization the L3 adjuvant was mixed with the LAM-TT conjugate in order to achieve a dose of 10 µg in 100 µl for parenteral injection or 10 µl for nasal administration.

Immunization 1; 0 weeks (parenteral for all groups). Immunization 2; 3 weeks (nasally for all groups except live BCG which was administered parenterally). Challange; 4 weeks

Changes of body weight (%) related to initial weight at time 0 weeks. Average body weight changes ± SE of 10 mice/group are shown in Fig.1.

As can be seen from the weight changes, both of the adjuvant formulations containing LAM-TT result in a positive body weight development.

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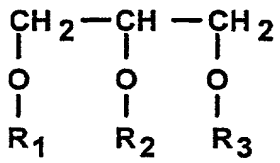
Claims

1. Vaccine formulation against a mycobacterium comprising,
as adjuvant, one or more substances selected from

a) monoglyceride preparations having at least 80 % monoglyceride content and having the general formula



wherein R_1 and R_2 is H and R_3 is one acyl group containing from 6 to 24 carbon atoms, and where the acyl chains may contain one or more unsaturated bonds, in admixture with one or more substances selected from

b) fatty acids of the general formula

$$CH_3 - (CH_2)_n - COOH$$

where "n" may be varied between 4 and 22, and where the acyl chain may contain one or more unsaturated bonds, and

as immunizing component, an immunogenic product consisting of antigenically active carbohydrate moieties (ACM) derived from *Mycobacterium tuberculosis* which are each covalently coupled, possibly via identical divalent bridge groups, to immunologically active carriers (IAC).

2. Vaccine formulation according to claim 1, wherein the immunologically active carriers (IAC) contain amino groups and said divalent bridge group has the following structural formula

$$(LAM)\text{-}NH\text{-}C\text{-}(CH_2)_3\text{-}S\text{-}CH_2\text{-}C\text{-}NH\text{-}(IAC).$$

(with NH_2Cl and O above the two C atoms)

3. Vaccine formulation according to claim 1 or 2, wherein the adjuvant has a monoglyceride preparation content of at least 90 %, preferably at least 95 %, and the acyl chains of the monoglyceride preparation contains 8 to 20 carbon atoms, preferably 14 to 20 carbon atoms, and the acyl chains optionally contains one or more unsaturated bonds, and the immunologically active carriers (IAC) are derived from

polypeptides and are selected from tetanus toxoid, diphtheria toxoid, cholera subunit B or Protein D from H. influenzae.

4. Vaccine formulation according to any one of claims 1 - 3, which further comprises pharmaceutical excipients selected from the group consisting of biocompatible oils, physiological saline solution, preservatives and osmotic pressure controlling agents, carrier gases, pH-controlling agents, organic solvents, hydrophobic agents, enzyme inhibitors, water absorbing polymers, surfactants, absorption promoters, and anti-oxidative agents.

5. Vaccine formulation according to claim 3 or 4, wherein the adjuvant is a mixture of mono-olein and oleic acid, and possibly soybean oil, and the immunizing component is lipoarabinomannan-tetanus toxoid (LAM-TT).

6. Vaccine formulation according to any one of claims 1-5, wherein the formulation is formulated into a preparation for mucosal administration.

7. Vaccine formulation according to claim 6, wherein the mucosal administration is selected from nasal, pulmonary, oral, rectal and vaginal administration.

8. Aerosol or spray package comprising a tuberculosis vaccine composition according to any one of the claims 1 - 7.

9. Nose-drop package comprising a tuberculosis vaccine composition according to any one of the claims 1 - 6.

10. A method of vaccinating a mammal against a mycobacterium having antigenically active carbohydrate moieties (ACM) derived from *Mycobacterium tuberculosis,* which comprises mucosal administration to the mammal of an protection-inducing amount of a tuberculosis vaccine composition according to any one of claims 1 - 7.

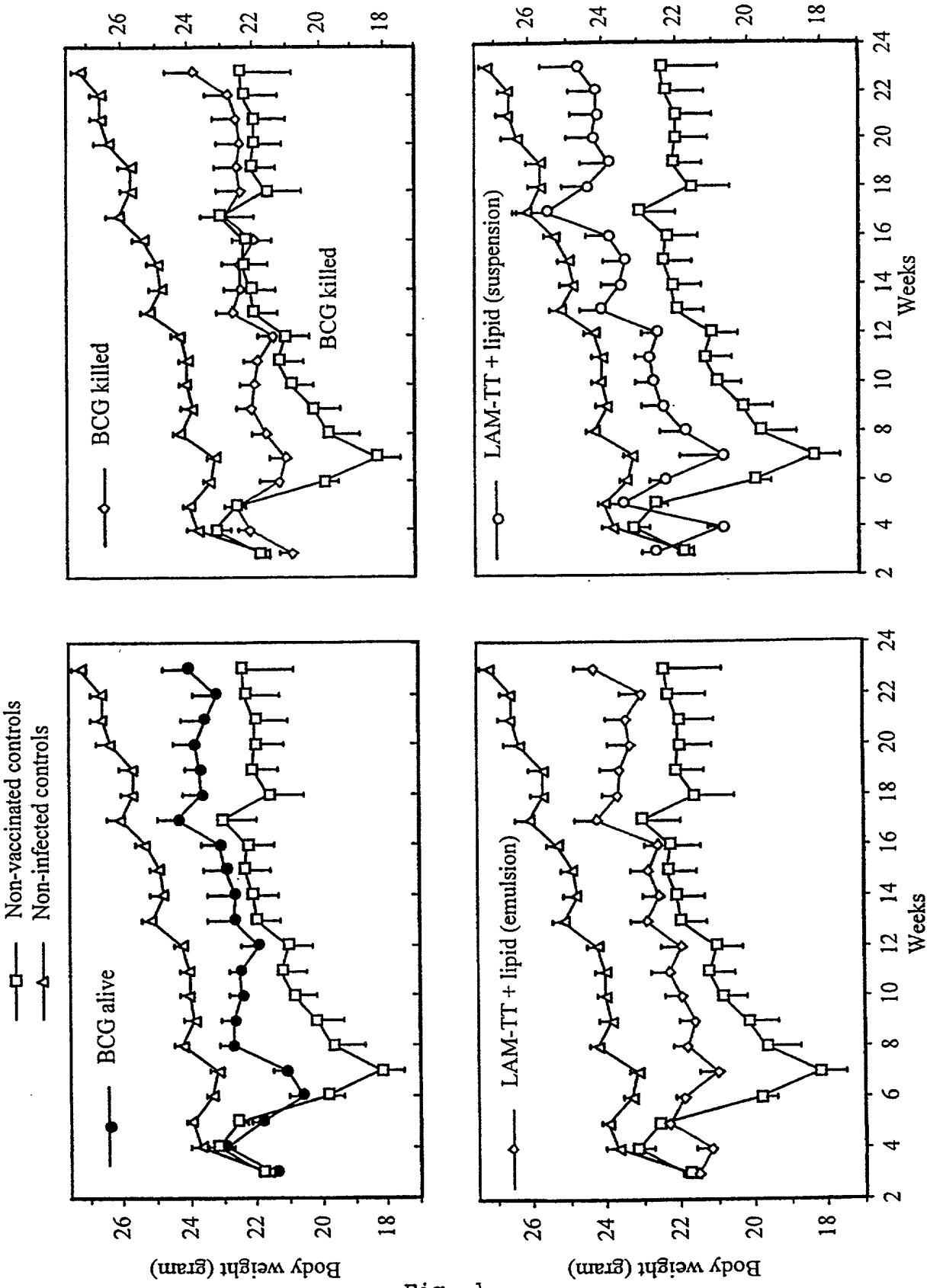

Non-vaccinated controls
Non-infected controls
BCG alive
BCG killed
BCG killed
LAM-TT + lipid (suspension)
LAM-TT + lipid (emulsion)
Body weight (gram)
Weeks

Fig. 1

ATTORNEY/DOCKET NO: **SCHR3002/REF**

DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention (Design, if applicable) entitled:

VACCINE FORMULATION

the specification of which (check one):

☐ is attached hereto, or ☒ was filed on: **9 February 2000** as PCT International Application Number: **PCT/EP00/01038**
and (if applicable) was amended on: **24 January 2001**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in *Title 37, Code of Federal Regulations, §1.56.* I hereby claim foreign priority benefits under *Title 35, United States Code §119* of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)			PRIORITY CLAIMED	
Number	**Country**	**Day/Month/Year Filed**	**Yes**	**No**
9900496-2	SE	12 February 1999	X	

☐ Additional Priority Application(s) Listed on Following Page(s)

I HEREBY CLAIM THE BENEFIT UNDER TITLE 35 U.S. CODE §119(E) OF ANY U.S. PROVISIONAL APPLICATIONS LISTED BELOW.	
Application Number	**Day/Month/Year Filed**

☐ Additional Provisional Application(s) Listed on Following Page(s)

I hereby claim the benefit under *Title 35, United States Code, §120* of any United States application(s) or PCT international application(s) designating The United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of *Title 35, United States Code, §112*, I acknowledge the duty to disclose information which is material to patentability as defined in *Title 37, Code of Federal Regulations, §1.56* which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Number	Filing Date	Status - Patented, Pending or Abandoned

☐ Additional US/PCT Priority Application(s) listed on Following Page(s)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under *section 1001 of title 18 of the United States Code* and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (We) hereby appoint as my (our) attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: J. Ernest Kenney, Reg. No. 19,179; Eugene Mar, Reg. No. 25,893; Richard E. Fichter, Reg. No. 26,382; Thomas J. Moore, Reg. No. 28,974; Joseph DeBenedictis, Reg. No. 28,502; Benjamin E. Urcia, Reg. No. 33,805; and

I(we) authorize my(our) attorneys to accept and follow instructions from AB Stockholms Patentbyra Zacco regarding any matter related to the preparation, examination, grant and maintenance of this application, any continuation, continuation-in-part or divisional based thereon, and any patent resulting therefrom, until I(we) or my(our) assigns withdraw this authorization in writing.

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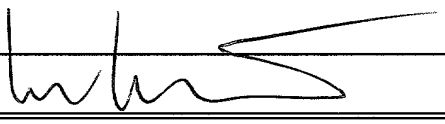
DATE Aug 13, 2007	SIGNATURE 

☒ *See following page(s) for additional joint inventors.*

(04AUG1998)

CONTINUATION OF DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

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DATE	SIGNATURE

FULL NAME OF JOINT INVENTOR	CITIZENSHIP
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